

WHAT IS CLAIMED IS:

1 1. An isolated infectious human-bovine chimeric parainfluenza virus
2 (PIV) comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a
3 large polymerase protein (L), and a partial or complete PIV background genome or
4 antigenome of a human PIV (HPIV) or bovine PIV (BPIV) combined with one or more
5 heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a different PIV to
6 form a human-bovine chimeric PIV genome or antigenome.

1 2. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) encodes one or more PIV N, P, C, D, V, M,
3 F, HN and/or L protein(s) or fragment(s) thereof.

1 3. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) encodes a complete open reading frame
3 (ORF) of one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s).

1 4. The chimeric PIV of claim 1, wherein said one or more
2 heterologous gene(s) or genome segment(s) includes a heterologous regulatory element
3 comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end
4 signal, RNA editing site, encapsidation signal, intergenic region, or 3' or 5' non-coding
5 region.

1 5. The chimeric PIV of claim 1, wherein said background genome or
2 antigenome incorporates a heterologous genome segment integrated with the background
3 genome or antigenome to form a chimeric gene.

1 6. The chimeric PIV of claim 1, wherein a heterologous gene or
2 genome segment is added adjacent to or within a noncoding region of the partial or
3 complete PIV background genome or antigenome.

1 7. The chimeric PIV of claim 1, wherein a heterologous gene or
2 genome segment is added or substituted at a position corresponding to a wild-type gene
3 order position of a counterpart gene or genome segment within the partial or complete
4 PIV background genome or antigenome.

8. The chimeric PIV of claim 1, wherein a heterologous gene or genome segment is added or substituted at a position that is more promoter-proximal or promoter-distal compared to a wild-type gene order position of a counterpart gene or genome segment within the partial or complete PIV background genome or antigenome.

9. The chimeric PIV of claim 1, wherein the chimeric genome or antigenome comprises a partial or complete HPIV background genome or antigenome combined with one or more heterologous genes or genome segments from a BPIV.

10. The chimeric PIV of claim 9, wherein the partial or complete HPIV background genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a BPIV to form a human-bovine chimeric PIV genome or antigenome.

11. The chimeric PIV of claim 9, wherein said one or more heterologous gene(s) or genome segment(s) encodes a complete open reading frame (ORF) of one or more PIV N, P, L, or M protein(s).

12. The chimeric PIV of claim 9, wherein a bovine PIV3 N, M, L, or P open reading frame (ORF) is substituted for a human PIV3 N, M, L, or P ORF to form the chimeric PIV.

13. The chimeric PIV of claim 9, wherein said one or more heterologous gene(s) or genome segment(s) includes a heterologous regulatory element comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end signal, editing region, intergenic region, or a 3' or 5' non-coding region.

14. The chimeric PIV of claim 9, wherein said heterologous gene or genome segment encodes a bovine PIV3 M, L, or P protein.

15. The chimeric PIV of claim 9, which is selected from rHPIV3-M_B, rHPIV3-L_B, or rHPIV3-P_B.

16. The chimeric PIV of claim 9, wherein the chimeric genome or antigenome is further modified by addition or substitution of one or more additional heterologous gene(s) or genome segment(s) from a bovine PIV within the partial or

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complete bovine background genome or antigenome to increase genetic stability or alter attenuation, reactogenicity or growth in culture of the chimeric virus.

17. The chimeric PIV of claim 1, wherein the genome or antigenome is further modified by introduction of one or more attenuating mutations identified in a biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA virus.

18. The chimeric PIV of claim 17, wherein the genome or antigenome incorporates at least one and up to a full complement of attenuating mutations present within PIV3 JS *cp45*.

19. The chimeric PIV of claim 17, wherein the genome or antigenome incorporates at least one and up to a full complement of attenuating mutations specifying an amino acid substitution in the L protein at a position corresponding to Tyr₉₄₂, Leu₉₉₂, or Thr₁₅₅₈ of JS; in the N protein at a position corresponding to residues Val₉₆ or Ser₃₈₉ of JS, in the C protein at a position corresponding to Ile₉₆ of JS, in the M protein at a position corresponding to residues Pro₁₉₉ of JS, in the F protein at a position corresponding to residues Ile₄₂₀ or Ala₄₅₀ of JS, in the HN protein at a position corresponding to residue Val₃₈₄ of JS, a nucleotide substitution a 3' leader sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS, and/or a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS.

20. The chimeric PIV of claim 17, wherein the genome or antigenome incorporates attenuating mutations from different biologically derived mutant PIVs.

21. The chimeric PIV of claim 17, wherein the genome or antigenome incorporates an attenuating mutation at an amino acid position corresponding to an amino acid position of an attenuating mutation identified in a heterologous, mutant negative stranded RNA virus.

22. The chimeric PIV of claim 17, wherein the genome or antigenome includes at least one attenuating mutation stabilized by multiple nucleotide changes in a codon specifying the mutation.

23. The chimeric PIV of claim 1, wherein the genome or antigenome comprises an additional nucleotide modification specifying a phenotypic change selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in immunogenicity.

24. The chimeric PIV of claim 23, wherein the additional nucleotide modification alters one or more of the PIV N, P, C, D, V, M, F, HN and/or L genes and/or a 3' leader, 5' trailer RNA editing site, encapsidation signal, and/or an intergenic region.

25. The chimeric PIV of claim 23, wherein one or more genes of the chimeric virus is deleted in whole or in part or expression of the genes is reduced or ablated by a mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters an amino acid specified by an initiation codon, or by introduction of one or more stop codons in an open reading frame (ORF) of the gene.

26. The chimeric PIV of claim 23, wherein a modification is introduced in the chimeric genome or antigenome comprising a partial or complete deletion of one or more C, D and/or V ORF(s) or one or more nucleotide change(s) that reduces or ablates expression of said one or more C, D and/or V ORF(s).

27. The chimeric PIV of claim 17, wherein the chimeric genome or antigenome is modified to encode a non-PIV molecule selected from a cytokine, a T-cell helper epitope, a restriction site marker, or a protein of a microbial pathogen capable of eliciting a protective immune response in a mammalian host.

28. The chimeric PIV of claim 1, wherein the bovine-human chimeric genome or antigenome comprises a partial or complete PIV vector genome or antigenome combined with one or more heterologous genes or genome segments encoding one or more antigenic determinants of one or more heterologous pathogens.

29. The chimeric PIV of claim 28, wherein said one or more heterologous pathogens is a heterologous PIV and said heterologous gene(s) or genome segment(s) encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or fragment(s) thereof.

1 30. The chimeric PIV of claim 28, wherein the vector genome or
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous PIV(s).

1 31. The chimeric PIV of claim 30, wherein said one or more
2 heterologous PIV(s) is/are selected from HPIV1, HPIV2, or HPIV3.

1 32. The chimeric PIV of claim 30, wherein the vector genome or
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous HPIV(s).

1 33. The chimeric PIV of claim 32, wherein the vector genome or
2 antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more
4 heterologous HPIV(s).

1 34. The chimeric PIV of claim 33, wherein the chimeric genome or
2 antigenome incorporates one or more gene(s) or genome segment(s) of a BPIV that
3 specifies attenuation.

1 35. The chimeric PIV of claim 31, wherein one or more HPIV1 or
2 HPIV2 gene(s) or genome segment(s) encoding one or more HN and/or F glycoprotein(s)
3 or antigenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated
4 within the partial or complete HPIV3 vector genome or antigenome.

1 36. The chimeric PIV of claim 35, wherein both HPIV1 genes
2 encoding HN and F glycoproteins are substituted for counterpart HPIV3 HN and F genes
3 to form a chimeric HPIV3-1 vector genome or antigenome which is further modified by
4 addition or incorporation of one or more gene(s) or gene segment(s) encoding one or
5 more antigenic determinant(s) of HPIV2 and one or more heterologous gene(s) or genome
6 segment(s) of a BPIV that specifies attenuation.

1 37. The chimeric PIV of claim 35, wherein a transcription unit
2 comprising an open reading frame (ORF) of an HPIV2 HN gene is added to or
3 incorporated within the chimeric HPIV3-1 vector genome or antigenome.

1 38. The chimeric PIV of claim 32, wherein a plurality of antigenic
2 determinants of multiple HPIVs are added to or incorporated within the partial or
3 complete HPIV vector genome or antigenome.

1 39. The chimeric PIV of claim 35, wherein the vector genome or
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous
3 pathogen is selected from measles virus, subgroup A and subgroup B respiratory
4 syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2 human
5 immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein
6 Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1 40. The chimeric PIV of claim 39, wherein said one or more
2 heterologous antigenic determinant(s) is/are selected from measles virus HA and F
3 proteins, subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins,
4 mumps virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2
5 human immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus
6 gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein
7 Barr Virus gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and
8 NS1 proteins, and alphavirus E protein, and antigenic domains, fragments and epitopes
9 thereof.

1 41. The chimeric PIV of claim 40, wherein the heterologous pathogen
2 is measles virus and the heterologous antigenic determinant(s) is/are selected from the
3 measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 42. The chimeric PIV of claim 41, wherein a transcription unit
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or
3 incorporated within a HPIV3 vector genome or antigenome.

1 43. The chimeric PIV of claim 40, which incorporates a gene or
2 genome segment from respiratory syncytial virus (RSV).

1 44. The chimeric PIV of claim 43, wherein the gene or genome
2 segment encodes a RSV F and/or G glycoprotein or immunogenic domain(s) or epitope(s)
3 thereof.

1 45. The chimeric PIV of claim 1, wherein the chimeric genome or
2 antigenome comprises a partial or complete BPIV background genome or antigenome
3 combined with one or more heterologous gene(s) or genome segment(s) from a human
4 PIV.

1 46. The chimeric PIV of claim 45, wherein one or more HPIV
2 glycoprotein genes selected from HN and F, or one or more genome segments encoding a
3 cytoplasmic domain, transmembrane domain, ectodomain or immunogenic epitope
4 thereof, is/are substituted for one or more counterpart genes or genome segments within
5 the BPIV background genome or antigenome.

1 47. The chimeric PIV of claim 1 which is a virus.

1 48. The chimeric PIV of claim 1 which is a subviral particle.

1 49. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 1 combined with a
4 physiologically acceptable carrier.

1 50. The method of claim 49, wherein the chimeric PIV is administered
2 in a dose of 10^3 to 10^7 PFU.

1 51. The method of claim 49, wherein the chimeric PIV is administered
2 to the upper respiratory tract.

1 52. The method of claim 49, wherein the chimeric PIV is administered
2 by spray, droplet or aerosol.

1 53. The method of claim 49, wherein the chimeric PIV and a second
2 attenuated PIV are administered simultaneously as a mixture.

1 54. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 9 combined with a
4 physiologically acceptable carrier.

1 55. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 17 combined with a
4 physiologically acceptable carrier.

1 56. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 23 combined with a
4 physiologically acceptable carrier.

1 57. A method for stimulating the immune system of an individual to
2 induce protection against PIV which comprises administering to the individual an
3 immunologically sufficient amount of the chimeric PIV of claim 28 combined with a
4 physiologically acceptable carrier.

1 58. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in
3 a physiologically acceptable carrier.

1 59. The immunogenic composition of claim 58, formulated in a dose of
2 10^3 to 10^7 PFU.

1 60. The immunogenic composition of claim 58, formulated for
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1 61. The immunogenic composition of claim 58, wherein the chimeric
2 PIV elicits an immune response against one or more virus(es) selected from HPIV1,
3 HPIV2 and HPIV3.

1 62. The immunogenic composition of claim 61, wherein the chimeric
2 PIV elicits an immune response against HPIV3 and another virus selected from HPIV 1,
3 HPIV2 and HPIV3.

1 63. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 9 in
3 a physiologically acceptable carrier.

1 64. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 17 in
3 a physiologically acceptable carrier.

1 65. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 23 in
3 a physiologically acceptable carrier.

1 66. An immunogenic composition to elicit an immune response against
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 28 in
3 a physiologically acceptable carrier.

1 67. An isolated polynucleotide molecule comprising a partial or
2 complete PIV background genome or antigenome of a human PIV (HPIV) or bovine PIV
3 (BPIV) combined with one or more heterologous gene(s) or genome segment(s) of a N, P,
4 L, or M gene of a different PIV to form a human-bovine chimeric PIV genome or
5 antigenome.

1 68. The isolated polynucleotide molecule of claim 67, wherein said one
2 or more heterologous gene(s) or genome segment(s) encodes one or more PIV N, P, L, or
3 M protein(s) or fragment(s) thereof.

1 69. The isolated polynucleotide molecule of claim 67, wherein said one
2 or more heterologous gene(s) or genome segment(s) encodes a complete open reading
3 frame (ORF) of one or more PIV N, P, L, or M protein(s).

1 70. The isolated polynucleotide molecule of claim 67, wherein said one
2 or more heterologous gene(s) or genome segment(s) includes a heterologous regulatory

element comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end signal, editing region, encapsidation signal, intergenic region, or 3' or 5' non-coding region.

71. The isolated polynucleotide molecule of claim 67, wherein the chimeric genome or antigenome comprises a partial or complete HPIV background genome or antigenome combined with one or more heterologous genes or genome segments from a BPIV.

72. The isolated polynucleotide molecule of claim 71, wherein the partial or complete HPIV background genome or antigenome is combined with one or more heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a BPIV to form a human-bovine chimeric PIV genome or antigenome.

73. The isolated polynucleotide molecule of claim 71, wherein said one or more heterologous gene(s) or genome segment(s) encodes a complete open reading frame (ORF) of one or more PIV N, P, L, or M protein(s).

74. The isolated polynucleotide molecule of claim 71, wherein a bovine PIV3 N, L, M or P open reading frame (ORF) is substituted for a human PIV3 N, L, M, or P ORF to form the chimeric PIV.

75. The isolated polynucleotide molecule of claim 71, wherein said one or more heterologous gene(s) or genome segment(s) includes a heterologous regulatory element comprising an extragenic 3' leader or 5' trailer region, a gene-start signal, gene-end signal, editing region, intergenic region, or a 3' or 5' non-coding region.

76. The isolated polynucleotide molecule of claim 71, wherein said heterologous gene or genome segment encodes a bovine PIV3 M, L, or P protein.

77. The isolated polynucleotide molecule of claim 76, wherein said heterologous gene or genome segment encodes a bovine PIV3 M protein.

78. The isolated polynucleotide molecule of claim 76, wherein said heterologous gene or genome segment encodes a bovine PIV3 P protein.

1 79. The isolated polynucleotide molecule of claim 76, wherein said
2 heterologous gene or genome segment encodes a bovine PIV3 L protein.

1 80. The isolated polynucleotide molecule of claim 67, wherein the
2 chimeric genome or antigenome is further modified by one or more attenuating
3 mutations.

1 81. The isolated polynucleotide molecule of claim 67, further
2 comprising a nucleotide modification specifying a phenotypic change selected from a
3 change in growth characteristics, attenuation, temperature-sensitivity, cold-adaptation,
4 plaque size, host-range restriction, or a change in immunogenicity.

1 82. A method for producing an infectious attenuated chimeric PIV
2 particle from one or more isolated polynucleotide molecules encoding said PIV,
3 comprising:

4 expressing in a cell or cell-free lysate an expression vector comprising an
5 isolated polynucleotide comprising partial or complete PIV background genome or
6 antigenome of a human PIV (HPiV) or bovine PIV (BPiV) combined with one or more
7 heterologous gene(s) or genome segment(s) of a N, P, L, or M gene of a different PIV to
8 form a human-bovine chimeric PIV genome or antigenome.

1 83. The method of claim 82, wherein the chimeric PIV genome or
2 antigenome and the N, P, L and/or M proteins are expressed by two or more different
3 expression vectors.

1 84. An expression vector comprising an operably linked transcriptional
2 promoter, a polynucleotide sequence which includes a partial or complete PIV
3 background genome or antigenome of a human PIV (HPiV) or bovine PIV (BPiV)
4 combined with one or more heterologous gene(s) or genome segment(s) of a N, P, L, or
5 M gene of a different PIV to form a human-bovine chimeric PIV genome or antigenome,
6 and a transcriptional terminator.